SUPPORT FOR THE AMENDMENTS AND NEW CLAIMS

The amendments are supported by the claims as originally filed, and support for the addition of procedure (c) in claim 40 can be found, for example, at page 64 lines 18-22 and page 65 lines 6-12. Thus, the amendments do not constitute new matter.

Supplemental Information Disclosure Statement

The Applicants note that a supplemental information disclosure statement, PTO form 1449, and 4 cited references were submitted with the response filed on September 26, 2006.

REMARKS

1. Rejection under 35 USC 102(e)

The Patent Office rejected claims 40 and 41 under 35 USC 102(e) as being anticipated by US 6,794,128 ("Marks"). The Applicants traverse this rejection.

In order to serve as an appropriate anticipatory reference under 35 USC 102(e), a reference must teach each and every limitation of the rejected claim. Currently pending claim 40 recites as follows:

> A machine readable storage medium comprising a program containing a set of instructions for causing a cell screening system to execute procedures for measuring internalization of cell surface receptor proteins in individual cells on an array of locations which contain multiple cells, wherein the procedures comprise:
>
> a) identifying internalized cell surface receptor proteins in multiple individual cells on the array of locations, wherein the individual cells comprise at least a first luminescent reporter molecule that reports on a cell surface receptor protein of interest, and at least a second luminescent reporter molecule that reports on cells, wherein the identifying comprises determining whether luminescent signals from the at least first luminescent reporter molecule in the individual cells identified by the at least second luminescent reporter molecule meet or surpass a user-defined threshold luminescent intensity, wherein luminescent signals from the at least first luminescent reporter molecule that meet or surpass the user-defined threshold luminescent intensity represent an internalized cell surface receptor protein;
>
> b) calculating a number and/or percent of the individual cells that internalized the at least first luminescently labeled reporter molecule wherein the calculations provide a measure of internalization of the cell surface receptor protein in the individual cells; and
>
> c) displaying data on internalized cell surface receptor proteins.

Marks does not teach at least the following limitations of claim 40

-Procedures for measuring internalization of cell surface receptor proteins;

> -Calculating a number and/or percent of the individual cells that internalized the at least first luminescently labeled reporter molecule wherein the calculations provide a measure of internalization of the cell surface receptor protein in the individual cells; and
> - Displaying data on internalized cell surface receptor proteins

Marks does teach labeling of antibodies or bacteriophage (ie: labeling of polypeptides that are **not** cell surface receptor proteins) and assessing their internalization by fluorescence microscopy. Contrary to the Patent Office's characterization, the disclosure of Marks at column 13 lines 44-55 does **not** teach methods for identifying internalizing receptors. Instead, this passage states as follows:

> "Identification of Internalizing Receptors
> Once an antibody or polypeptide that is internalized into a cell has been identified, it is possible to probe one or more cell types with the identified antibody or polypeptide to identify the target recognized and bound by the antibody. Since the antibody is an internalizing antibody it is ***likely*** that such targets are themselves internalizing targets (e.g. members or portions of internalizing receptors)"

Thus, Marks disclosure simply teaches that, **if** the antibody or bacteriophage happens to bind to a receptor and, **if** after binding that receptor happens to internalize together with the antibody or bacteriophage (thus requiring that they stay bound, which is often not the case), then bound and internalized receptor can be identified. This interpretation is supported by the disclosure in Column 1, lines 20-25 cited by the patent office (e.g.: "...as well as the internalizing receptors **bound**.") This is quite distinct from the presently claimed invention.

Furthermore, the Patent Office's reliance on the Marks disclosure at column 46 lines 47-48; column 47 line 65 to column 48 line 3; and Figure 9 to assert that Marks teaches providing a measure of receptor internalization is misplaced. These passages refer to a measure of GFP **expression** from SKBR3 cells after incubation with F5-GFP phagemids. F5 is disclosed as an "anti-ErbB2-scFV" (see column 42 line 64) (See also the description of Figure 9 at column 11 lines 21-39). Thus, the passage cited by the Patent Office teaches expression of a GFP-antibody chimeric protein in cells—this is **clearly not** a teaching of a measure of cell surface receptor protein internalization.

Based on the above, it is clear that Marks does not teach all (or even many) of the limitations of claim 40, and thus is not a proper anticipatory reference. Since claim 41 is dependent on claim 40, it contains all of the limitations of claim 40, and thus Marks is not a proper anticipatory reference for claim 41.

Therefore, the Applicants respectfully request reconsideration and withdrawal of the rejection.

2. Rejections under 35 USC Section 112, first paragraph

The Patent Office rejected claims 40-43 under 35 USC 112 first paragraph, as failing to comply with the written description requirement, based on the assertion that the claimed subject matter was not described to reasonably convey to those of skill in the art that the inventors had possession of the claimed invention at the time of filing the application. The Applicants traverse this rejection.

(a) The Patent Office asserted that the specification does not provide adequate written description for the recitation in claim 40 of cells contain two luminescent reporter molecules, one which identifies the cell and another which reports on an internalizing cell surface receptor. As noted by the Patent Office, the Applicants pointed to Example 4, pages 64-67 as providing support. However, the Patent Office asserted that the Example "does not illustrate the reporting of internalizing cell surface receptors." The Patent Office provides no support for this assertion other than this statement, and the Applicants traverse this assertion.

Example 4 is entitled "High-content screen of ligand-induced parathyroid hormone **receptor internalization**." **(See page 60, lines 5-6)** Example 4 continues as follows:

> Plasmid construct. A eukaryotic expression plasmid containing the coding sequence for a humanized GFP mutant (pEGFP-N_2, CLONTECH, Palo Alto, CA) was used to create a **GFP-human parathyroid hormone receptor (PTHR, GenBank #L04308) chimera**. *(This describes a recombinant protein between a luminescent reporter protein (GFP) and a cell surface receptor protein (PTHR—a cell surface receptor protein that is the receptor for parathyroid hormone; thus, this is a luminescent reporter molecule that reports on an internalizing cell surface receptor protein)* **(See page 60, lines 7-10)**
>
> Cell preparation. **The plasmid construct was used to transfect a human embryonic kidney cell line** (HEK 293). *(This describes cells in which receptor internalization can be reported on by the luminescent reporter molecule that reports on an internalizing cell surface receptor)* **(See page 60, lines 11-12)**
>
> **Parathyroid hormone induction of GFP-PTHR internalization**. A 100uM stock of bovine parathyroid hormone (PTH), amino acids 1-34 (Bachem, King of Prussia, PA), is prepared using acidified water (pH 4-4.5). **To induce internalization of the GFP-PTHR chimera, cells are stimulated by the addition of 50ul of 500 nM PTH to each well** *(This describes internalization*

into the cell of the luminescent reporter molecule that reports on the cell surface receptor protein.) **(See page 60 lines 21 to page 61 line 1)**

Following the two hour PTH stimulation, the media is decanted from the plate and **the cells are fixed and the nuclei stained by the addition of 200ul of Hank's Balanced Salt Solution (HBSS) containing 3.7% formalin (Sigma) and 1ug/ml Hoechst 33342 (Molecular Probes, Eugene, Oregon)**. *(This describes contact of the cells with a luminescent reporter that identifies the cells (Hoechst 33342))* **(See page 61, lines 5-8)**

Based on the above, it is clear that Example 4 provides very clear support for individual cells that comprise at least a first luminescent reporter molecule that reports on a cell surface receptor protein of interest, and at least a second luminescent reporter molecule that identifies cells. Therefore, the Applicants respectfully request reconsideration and withdrawal of this rejection.

(b) The Patent Office asserted that the specification does not provide adequate written description for the recitation in claims 42-43 of steps involving "calculating normalized aggregate intensities, the area, the number of objects that represent internalized cell surface receptors, and obtaining high and low resolutions." The Applicants traverse this assertion.

Referring again to Example 4, the specification discloses as follows:

Image acquisition and analysis. (See **Figure 26** for overview) After autofocusing 101 (**Figure 27**) on the **Hoechst-labeled nuclei,** an image of the nuclei 102 is acquired at 20x magnification...**An image of the GFP fluorescence is then acquired at 20x magnification** 105. **(Figure 26)** *(This describes that tthe GFP fluorescence is the signal from the luminescent reporter molecule that reports on the internalizing cell surface receptor* **(See page 61, lines 13-19)**

The object image is processed to determine which bright spots represent the internalized receptor in stimulated cells. This process uses a brightness threshold and a minimum size set by the user. The object image is thresholded at the brightness threshold to create the binary object mask 108. **(Figure 26)** The objects in the binary object mask are labeled and their sizes **are** measured in pixels. **Those objects that meet or exceed the minimum size are valid spots** 109; **(Figure 28)** the rest are ignored. (*This describes that the "spots" represent validated internalized receptors)* **(See page 62 line 19 to page 63 line 2)**

Example 4 goes on as follows **(See page 63 line 3 to page 66 lines 19)**

The following measurements are then determined **for each valid spot**. **(Figure 28)** **The count of spots in the field is incremented** 110. The number of pixels was previously counted. For each valid spot, the region with its label is extracted from the binary object mask to create the single-spot binary mask. The single-spot binary mask is applied to the original object image to get the grayscale spot image of the respective spot. The intensities of the pixels in the grayscale spot image are summed to get the **aggregate intensity of the spot** 111. Once all the spots have been processed, **the sum of all of the areas of the valid spots are summed to get the aggregate spot area for the field** 112. **The aggregate intensity of the spots is totaled to get the aggregate spot intensity of the field. There are several statistics to choose from for the final score for the field (or well): (a) the number of valid spots; (b) the aggregate area of the valid spots; (c) the aggregate intensity of the valid spots; (d) the aggregate intensity of the valid spots divided by the total area of the nuclei. When more than one field is analyzed within each well, the values for all the fields of the well are averaged together to get an aggregate statistic for the well** 113. **(Figure 26)**

The following examples of determining receptor internalization using the above techniques illustrate the differences found between treated and untreated cells. The nuclei of unstimulated cells are labeled with the DNA-specific Hoechst stain and imaged with a near-UV fluorescence filter set. The same cells are imaged with a blue fluorescence filter set which shows the distribution of the GFP fluorescence. The nuclear mask is derived by applying a threshold to the nucleus-labeled image, and the background image is derived by the grayscale erosion and dilation of the GFP image, showing the variations in the background intensity. The object image is then derived by subtracting the background image from the GFP image, resulting in faint spots. The object mask is then derived by applying the threshold to the object image. Some faint spots are eliminated by the thresholding. Some others have fewer pixels over the threshold than the requirement for a valid spot. **As a result, very few valid spots are found in the image of unstimulated cells. The spot count, aggregate spot areas, and aggregate spot brightness all have low values**.

In a second example, the nuclei of stimulated cells are labeled with the DNA-specific Hoechst stain and imaged as in the preceding example. The nuclear mask is derived by the automated thresholding method, and the background image is derived by the grayscale erosion and dilation of the GFP image, showing the variations in the background intensity. The object image is derived by subtracting the background image from the GFP image, resulting in bright spots. The object mask is derived by applying the threshold to the object image. **Many spots are seen in the object mask, and many of those spots have enough pixels over the threshold to meet the requirement for valid spots. The spot count, aggregate spot areas, and aggregate spot brightness all have high values.** Results from experiments like these examples were shown previously in Figure 25.

Figure 29 shows a representative display of a PC screen showing data which was obtained by the methods described in the above examples. **Each data point represents the Spot Count of a single well of the plate, calculated by summing together the Spot Counts of the fields of the well.** The graph 300 shows individual curves, each representing a single row of the 96 well plate. The leftmost six points of each curve represent the Spot Counts of untreated wells,

while the rightmost six points represent treated wells. The Spot Count feature ("obj count" in illustration) can be selected using the list 302. The numerical values for all the rows are shown in spreadsheet format 303. The graph 300 and spreadsheet 303 can be printed, and the spreadsheet can be exported in a comma-separated format for input into a spreadsheet program such as Microsoft Excel ™.

Alternatively, the data can be displayed on a field by field basis (**Figure 30**). Each graph at the top 304, 305, and 306 can be set to plot any one of the computed statistics (averaged over the fields of the well) vs. the well number. The spreadsheet 307 shows the numerical data computed on a field by field basis. Selection of a line from the spreadsheet causes display of the corresponding Hoechst 308 and GFP 309 images to be displayed. The spreadsheet 305 can be printed or exported in an ASCII file format for input into a spreadsheet program such as Microsoft Excel ™.

The graph 304 shows the **Spot Count vs. the well number**. **The Spot Count is the number of valid spots detected in the input GFP images**. The invention provides a computer means for converting the digital signal from the camera into this parameter and for plotting the parameter vs. the well number.

The graph 305 shows the **aggregate spot area ("total spot area" in illustration) vs. the well number**. **The aggregate spot area is the summed areas of all valid spots detected in the input GFP images**. The invention provides a computer means for converting the digital signal from the camera into this parameter and for plotting this parameter vs. the well number.

The graph 306 shows the **normalized spot intensity ratio ("Spot Inten Ratio x 100" in illustration) vs. the well number. The normalized spot intensity ratio is the summed intensities of all the pixels in the valid spots detected in the input GFP images, divided by the summed number of pixels in the nucleus masks in the corresponding Hoechst image.** The invention provides a computer means for converting the digital signal from the camera into this parameter and for plotting the parameter vs. the well number.

Figure 25 is a graphical representation of data from validation runs of the **PTHR internalization screen**. The figure illustrates that the data for min. ("minimum response" = unstimulated) and max. ("maximum response" = stimulated) are consistent between different plates (the differences are not statistically significant), giving c.o.v.'s (coefficients of variance) within a consistent and acceptable range.

In a specific example of a high-content screen, four fields were acquired in each well. **The Spot Count was summed across the fields of a well, and averaged among the similarly treated wells**. The untreated half of the plate had a Spot Count of 69.3 ± 17.7 (mean ± Standard Deviation) times the untreated half of the plate, giving a Coefficient of Variation (COV, the Standard Deviation divided by the mean) of 26%. The values from the fields of the treated half of the plate had a Spot Count of 404.2 ± 41.2, giving a COV of (10%). The mean Spot Count of the treated half was 5.83 times the mean Spot Count of the untreated half.

The above sections from Example 4 clearly demonstrate that the specification provides ample written description for steps involving "calculating normalized aggregate intensities, the

area, and the number of objects that represent internalized cell surface receptors."

Furthermore, support for claims that recite "obtaining high and low resolutions" can be found for example, as follows:

> In another aspect of the invention, **combined high throughput and high content methods** and associated computer readable storage medium are provided for identifying compounds that induce or inhibit internalization of cell surface receptor proteins. In this aspect, cells are treated with a ligand for the receptor protein of interest, which produces a detectable signal upon stimulation of the receptor protein. The cells are then treated with the test compound, and then scanned in a high throughput mode to identify those cells that exhibit the ligand-induced detectable signal. Subsequently, only those cells that exhibited the detectable signal are scanned in a high content mode, to determine whether the test compound has induced internalization of the luminescently labeled cell surface receptor protein into the cell. **(See page 10 line 16 to page 11 line 3)**
>
> The following example is a screen for activation of a G-protein coupled receptor (GPCR) as detected by the translocation of the GPCR from the plasma membrane to a proximal nuclear location. This example illustrates how a **high throughput screen can be coupled with a high-content screen** in the dual mode System for Cell Based Screening. **(See page 58 line 21 to page 59 line 2)**
>
> Figure 19 illustrates a dual mode screen for activation of a GPCR. Cells carrying a stable chimera of the GPCR with a blue fluorescent protein (BFP) are loaded with the acetoxymethylester form of Fluo-3, a cell permeable calcium indicator (green fluorescence) that is trapped in living cells by the hydrolysis of the esters. They are then deposited into the wells of a microtiter plate 601. The wells are then treated with an array of test compounds using a fluid delivery system, and a short sequence of Fluo-3 images of the whole microtiter plate are acquired and analyzed for wells exhibiting a calcium response (i.e., high throughput mode). The images appear like the illustration of the microtiter plate 601 in Figure 19. A small number of wells, such as wells C4 and E9 in the illustration, would fluoresce more brightly due to the Ca^{++} released upon stimulation of the receptors. **The locations of wells containing compounds that induced a response 602, would then be transferred to the HCS program and the optics switched for *detailed* cell by cell analysis** of the blue fluorescence for evidence of GPCR translocation to the perinuclear region. The bottom of Figure 19 illustrates the two possible outcomes of **the analysis of the high resolution cell data**. The camera images a sub-region 604 of the well area 603, producing images of the fluorescent cells 605. In well C4, the uniform distribution of the fluorescence in the cells indicates that the receptor has not internalized, implying that the Ca^{++} response seen was the result of the stimulation of some other signaling system in the cell. The cells in well E9 606 on the other hand, clearly indicate a concentration of the receptor in the perinuclear region clearly indicating the full activation of the receptor. **Because only a few hit wells have to be analyzed with high resolution, the overall throughput of the dual mode system can be**

quite high, comparable to the high throughput system alone. *(This clearly describes that the high content screen, only of positives from the high throughput screen) provides a higher resolution image than the high throughput screen)* **(See page 59 line 3 to page 60 line 3)**

The above sections clearly demonstrate that the specification provides ample written description for steps involving "obtaining high and low resolution images."

Based on all of the above, the Applicants respectfully request reconsideration and withdrawal of the rejections.

3. Rejections under 35 USC Section 112, second paragraph

(a) The Patent Office rejected claims 40-43 based on the assertion that the recitation of "internalized" cell surface receptors on the first line of limitation (a) in claim 40 is indefinite. Specifically, the Patent Office asserts that it is unclear whether "internalized defines the state of the cell surface protein or the name," and thus whether the phrase includes cell surface receptors that "can be internalized." The Applicants traverse this rejection.

The clause at issue in Claim 40 is as follows:

> A machine readable storage medium comprising a program containing a set of instructions for causing a cell screening system to execute procedures for ***measuring internalization of cell surface receptor proteins*** in individual cells on an array of locations which contain multiple cells, wherein the procedures comprise:
>
> a) ***identifying internalized cell surface receptor proteins*** in multiple individual cells on the array of locations.....

A cell surface receptor protein is only internalized if it has been internalized—one that is "capable" of being internalized is not "internalized," (at best, it is "internalizable"). Thus, it would be quite clear to those of skill in the art that the phrase in question on the first line of procedure (a) of claim 40 refers to cell surface proteins that have been internalized, as any other reading would be contrary to the definition of "internalized."

Therefore, the Applicants respectfully request reconsideration and withdrawal of the rejection.

(b) The Patent Office rejected claims 40-43 based on the assertion that the recitation of "identifies" in Claim 40, procedure (a) with respect to the function of the

second luminescent reporter molecule rendered the claim indefinite. The Applicants traverse this rejection, but have nonetheless amended the claim to obviate the rejection.

4. Claim rejections under 35 USC 101

The Patent Office rejected claims 40-43 as directed to non-statutory subject matter, based on the assertion that the claims "do not produce a result which meets the standard of being concrete, tangible, and useful." The Applicants traverse this rejection.

Claim 40 recites as follows:

> A machine readable storage medium comprising a program containing a set of instructions **<u>for causing a cell screening system to execute procedures</u>** for measuring internalization of cell surface receptor proteins in individual cells on an array of locations which contain multiple cells, **<u>wherein the procedures comprise</u>**:
>
> a) identifying internalized cell surface receptor proteins in multiple individual cells on the array of locations, wherein the individual cells comprise at least a first luminescent reporter molecule that reports on a cell surface receptor protein of interest, and at least a second luminescent reporter molecule that reports on cells, wherein the **<u>identifying comprises determining whether luminescent signals from the at least first luminescent reporter molecule in the individual cells identified by the at least second luminescent reporter molecule meet or surpass a user-defined threshold luminescent intensity</u>**, wherein luminescent signals from the at least first luminescent reporter molecule that meet or surpass the user-defined threshold luminescent intensity represent an internalized cell surface receptor protein; and
>
> b) calculating a number and/or percent of the individual cells that internalized the at least first luminescently labeled reporter molecule wherein the calculations provide a measure of internalization of the cell surface receptor protein in the individual cells.

Thus, the claims clearly recite that the computer readable storage medium contains instructions for **<u>causing a cell screening system to execute procedures</u>** for measuring cell surface receptor protein internalization, and then goes on the recite those procedures. Thus, the claims are **<u>not</u>** limited to solely mathematical operations, but **<u>do</u>** include a physical transformation outside of a computer, by causing a cell screening system to execute the recited procedures. This is very clearly statutory subject mater. Nonetheless, in order to expedite

prosecution of the application, the Applicants have amended claim 40 as suggested by the Examiner to obviate the rejection. Thus the Applicants respectfully request reconsideration and withdrawal of this rejection.

Based on all of the above, the Applicants believe the claims are now allowable. If there are any questions or comments regarding this response, the Examiner is encouraged to contact the undersigned attorney as indicated below.

Respectfully submitted,

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